

Effects of elevated atmospheric CO₂ in agro-ecosystems on soil carbon storage

H. ALLEN TORBERT,* HUGO H. ROGERS,† STEPHEN A. PRIOR,†
WILLIAM H. SCHLESINGER‡ and G. BRETT RUNION§

*USDA-ARS, Grassland, Soil and Water Research Laboratory, 808 East Blackland Rd., Temple, TX 76502, USA, †USDA-ARS National Soil Dynamics Laboratory, Box 3439, Auburn, AL 36831–3439, USA, ‡Duke University, Box 90340, Durham, NC 27708–0340, USA, §School of Forestry, Auburn University, Auburn, AL 36849, USA

Abstract

Increasing global atmospheric CO₂ concentration has led to concerns regarding its potential effects on the terrestrial environment. Attempts to balance the atmospheric carbon (C) budget have met with a large shortfall in C accounting ($\approx 1.4 \times 10^{15}$ g C y⁻¹) and this has led to the hypothesis that C is being stored in the soil of terrestrial ecosystems. This study examined the effects of CO₂ enrichment on soil C storage in C3 soybean (*Glycine max* L.) Merr. and C4 grain sorghum (*Sorghum bicolor* L.) Moench. agro-ecosystems established on a Blanton loamy sand (loamy siliceous, thermic, Grossarenic Paleudults). The study was a split-plot design replicated three times with two crop species (soybean and grain sorghum) as the main plots and two CO₂ concentration (ambient and twice ambient) as subplots using open top field chambers. Carbon isotopic techniques using $\delta^{13}\text{C}$ were used to track the input of new C into the soil system. At the end of two years, shifts in $\delta^{13}\text{C}$ content of soil organic matter carbon were observed to a depth of 30 cm. Calculated new C in soil organic matter with grain sorghum was greater for elevated CO₂ vs. ambient CO₂ (162 and 29 g m⁻², respectively), but with soybean the new C in soil organic matter was less for elevated CO₂ vs. ambient CO₂ (120 and 291 g m⁻², respectively). A significant increase in mineral associated organic C was observed in 1993 which may result in increased soil C storage over the long-term, however, little change in total soil organic C was observed under either plant species. These data indicate that elevated atmospheric CO₂ resulted in changes in soil C dynamics in agro-ecosystems that are crop species dependent.

Keywords: carbon cycle, carbon dioxide, $\delta^{13}\text{C}$, *Glycine max*, soil carbon dynamics, *Sorghum bicolor*

Received 24 August 1996; revision accepted 3 January 1997

Introduction

The rise of CO₂ in the atmosphere is well documented (Keeling *et al.* 1989); what has not been documented are the sinks for this C. With 5.5×10^{15} g C y⁻¹ emitted from fossil fuel and 1.1×10^{15} g C y⁻¹ from land-use change (tropical deforestation), 5.2×10^{15} g C y⁻¹ are accounted for by atmospheric increase and by oceanic uptake, leaving an unknown sink of 1.4×10^{15} g C y⁻¹ (Schimel *et al.* 1995). One hypothesis that has been forwarded is that C is being stored in terrestrial ecosystems (Tans *et al.* 1990; Fisher *et al.* 1994) as a result of higher plant productivity induced by elevated CO₂. Increases in plant growth are well documented experimentally (Rogers &

Dahlman 1991; Rogers *et al.* 1994; Goudriaan & Zadoks 1995). Carbon fixed within biomass ultimately enters the soil where it may reside for hundreds of years (Parton *et al.* 1986; Wallace *et al.* 1990).

The ability of the soil to store C, however, is a highly debated scientific question. Changes in plant morphology (Thomas & Harvey 1983; Prior *et al.* 1995), physiology (Amthor 1991; Rogers & Dahlman 1991; Amthor *et al.* 1994; Rogers *et al.* 1994), and phytochemistry (Lekkerkerk *et al.* 1990; Liljeroth *et al.* 1994) as a result of increasing levels of atmospheric CO₂ will likely have dramatic impacts on plant–microbe interactions and, thus, on C cycling and the potential for C storage in soil. Schlesinger (1986, 1990) found little evidence for soil C storage. Lamborg *et al.* (1983) have argued that increased soil

Correspondence: H.A. Torbert, fax +1 817-7706561, e-mail Torbert@brcsun0.tamu.edu

microbial activity due to greater biomass C inputs in an elevated CO₂ environment will lead to increased soil organic matter decomposition and therefore atmospheric CO₂ enrichment would not result in accumulation of soil organic C. Alternatively, Goudriaan & de Ruiter (1983) proposed that increased soluble, easily decomposed C inputs (as a consequence of CO₂ enrichment) would accentuate substrate preferences among soil microbes. They further speculated that preference for easily decomposable substrates would retard the decomposition of recalcitrant plant debris and native soil organic matter. The end result would be an accumulation of soil organic matter. Experimental evidence put forth by Lekkerkerk *et al.* (1990) has supported these contentions with wheat grown under CO₂-enriched conditions in a short-term experiment.

Soil plays a major role in the global accounting of C not only because of the large amount of C stored in soil, with estimates ranging from 1395 to 1636 × 10¹⁵ g (Ajtay *et al.* 1979; Post *et al.* 1992; Schlesinger 1984), but also because the soil contribution to the annual flux of CO₂ to the atmosphere is 10 times that contributed by fossil fuel burning (Post *et al.* 1990). It has been roughly estimated that the 'pioneer agriculture effect' released some 60 × 10¹⁵ g C to the atmosphere from 1860 to 1890 (Wilson 1978). This is 1.5 times the amount of C emitted by all industrial (mainly fossil) sources prior to 1950.

Jackson (1992) estimated that 1.3 × 10¹⁵ g of gross CO₂ is removed from the atmosphere by crops each y. Agro-ecosystems are important in the global context not only because of the CO₂ fluxes contributed to the atmosphere by these systems, but also because C storage in agro-ecosystems could be very sensitive to management practices (tillage and cropping systems) (Kern & Johnson 1993). All these factors combined make the understanding of the C cycling in agro-ecosystems important. The objective of this study was to examine the effect of elevated atmospheric CO₂ on soil C storage in both C3 and C4 agro-ecosystems.

Materials and methods

This study was conducted in an outdoor soil bin at the USDA-ARS National Soil Dynamics Laboratory in Auburn, AL. The bin, 2-m deep, 7-m wide, and 76-m long, was uniformly filled with the surface soil of a Blanton loamy sand (loamy, siliceous, thermic Grossarenic Paleudult) that had been continuously fallow for more than 25 years (Batchelor JA 1984 Properties of bin soils at the National Soil Dynamics Laboratory). The bottom of the bin was covered with sand and gravel and was tile drained. Initial levels of phosphorus (8 kg ha⁻¹) and potassium (14 kg ha⁻¹) were in the 'very low' range. Cation-exchange capacity averaged 2.45 cmol_c kg⁻¹, and

soil pH averaged 4.7. The initial level of organic matter averaged 5.0 g kg⁻¹ and total N was 0.06 g kg⁻¹. A more detailed description of the soil status prior to initiation of the study, fertilizer and lime amendments during the study, and subsequent soil analyses have been reported (Reeves *et al.* 1994). Initial fertilizer and lime additions had brought the soil nutrient status in to an acceptable range for crop production by the time of planting (Reeves *et al.* 1994).

The study was a split-plot design replicated three times with main plots of two plant species, and three CO₂-exposure regimes as subplots. Soybean and grain sorghum, were chosen as test crops to provide legume and non-legume crop species, respectively. The CO₂ exposure regimes were two open top field chambers at ambient and twice ambient atmospheric CO₂ concentrations and an open plot (no chamber) under ambient atmospheric CO₂ conditions.

The open top field chambers, 3-m in diameter and 2.4-m high, are described in detail by Rogers *et al.* (1983). Carbon dioxide concentrations were continuously monitored using a time-shared manifold with samples drawn through solenoids to an infrared CO₂ analyser (Model 6252, LI-COR, Inc., Lincoln, NE)¹. The atmospheric CO₂ concentration in the twice ambient chamber was continuously adjusted by injection of pure CO₂ into plenum fan boxes which dispensed air into each chamber through the bottom half of each chamber. The bottom half of the chamber cover was double-walled with the inside wall perforated with 2.5-cm diameter holes to serve as ducts to distribute air uniformly into the chamber. The average CO₂ concentrations were 357.5 ± 0.1 (mean ± SE) and 364.0 ± 0.2 µL L⁻¹ for ambient chambers and 705.0 ± 0.3 and 731.7 ± 0.4 µL L⁻¹ for twice ambient in 1992 and 1993, respectively. An open plot (no chamber) provided a control to test the effect of the open top chambers *per se* vs. the effects of CO₂ on plant response. The average CO₂ concentrations in the open plots were 357.4 ± 0.1 and 364.0 ± 0.1 µL L⁻¹, in 1992 and 1993, respectively.

Soybean 'Stonewall' and grain sorghum 'Savannah 5' were planted in 76-cm rows orientated across the width (perpendicular to long axis) of the soil bin on 2 June 1992 and 5 May 1993. Plants were thinned to a uniform density of 30 plants m⁻² for soybean and 26 plants m⁻² for grain sorghum.

To ensure adequate plant establishment, fertilizer N as NH₄NO₃ was broadcast applied at a rate of 34 kg N ha⁻¹ to both grain sorghum and soybean shortly after planting (4 June 1992 and 6 May 1993). In the grain sorghum, an additional 67 kg N ha⁻¹ was applied 30 days after planting (3 July 1992 and 7 June 1993).

¹Trade names and products are mentioned solely for information. No endorsement by USDA is implied

Following physiological maturity, plants within the whole plot were harvested for determination of total above-ground production (dry weight). The final harvest was 27 October for both crops in 1992, and on 1 October for grain sorghum and 5 October for soybean in 1993. Grain sorghum heads and soybean pods were removed from plants and processed through a plot combine. Plant stalks were cut into \approx 15-cm lengths using hedge clippers and uniformly spread over plots. Soybean pod hulls and grain sorghum chaff were added back to the plots with no plant residue incorporation. To simulate seed loss during combining, 10% (by weight) of the seed yield was returned to the plots. Bird netting (1.6 cm \times 1.9 cm opening; Dalen Products Inc., Knoxville, TN) was placed over the entire soil bin to prevent movement of above-ground residue into or out of plots. In addition, root samples were collected at physiological maturity by a root extraction technique (Bohm 1979). Root samples were collected from 12 and 16 plants per plot in 1992 and 1993, respectively.

Plant samples collected at physiological maturity were dried at 55 °C (to constant mass) and ground in a Wiley mill to pass a 0.44-mm screen. Six soil cores were collected from each chamber at harvest each year to a depth of 30 cm and bulked by depth increments of 0–5, 5–10, 10–15, 15–30 cm. Soil samples for the same soil depth increments were also collected before the atmospheric CO₂ enrichment treatment was initiated. Intact soil cores were collected for bulk density determination for the same depth increments.

Soil samples were analysed for soil organic matter carbon (SOMC) fractions using the procedures of Cambardella & Elliot (1992). The SOMC fractions were isolated by dispersing soil samples in 5 g L⁻¹ hexameta-phosphate and passing the dispersed soil through a 53- μ m sieve, with (as defined by Cambardella & Elliot 1992) particulate organic matter carbon (POMC) being retained on the sieve and the mineral associated carbon (MinC) passing through. However, with the Blanton loamy sand, the POMC consisted primarily of charcoal and most of the remaining carbon was found in the MinC pool carbon. The presence of charcoal in substantial quantities complicates the normal interpretation of POMC (Cambardella & Elliot 1992) and will therefore be referred to as charcoal.

The C concentration of plant and soil (SOMC, MinC, and charcoal) samples were determined using an elemental analyser (NA1500, Carlo Erba Instrumentazione, Milan, Italy). Carbon analysis on the charcoal soil fraction was calculated by difference in the total SOMC and the MinC soil fraction. Soil C concentration of each of the soil C fractions and bulk densities at each soil depth were used to calculate soil C content.

Air, plant, and soil (SOMC, charcoal, and MinC)

Table 1 The effect of plant species and atmospheric CO₂ content on $\delta^{13}\text{C}$ content of root material in 1992 and 1993.

		1992	1993
Grain Sorghum	Ambient	-12.8 (0.6)	-13.2 (0.5)
	Elevated	-32.6 (3.7)	-34.4 (0.4)
Soybean	Ambient	-29.7 (3.0)	-26.9 (0.7)
	Elevated	-45.4 (0.9)	-47.2 (2.1)

†Values represent means of three replicates. Standard deviation given in parentheses.

samples were measured for $\delta^{13}\text{C}$ content on a SIRA Series II isotope ratio mass spectrometer (VG ISOGAS, Middlewich, UK) after combustion in an elemental analyser. The $\delta^{13}\text{C}$ content of the charcoal soil fraction was performed by physically picking the charcoal pieces out of the soil for analysis. Isotopes of C, with $\delta^{13}\text{C}$ defined as:

$$\delta^{13}\text{C} = \left[\frac{^{13}\text{C}/^{12}\text{C}_{\text{sample}} - ^{13}\text{C}/^{12}\text{C}_{\text{standard}}}{^{13}\text{C}/^{12}\text{C}_{\text{standard}}} \right] \times 1000$$

(with a standard of Pee Dee belemnite) were used as a natural tracer of atmospheric CO₂ into the soil system (Boutton 1991a). The CO₂ source used for experimental enrichment was from combustion of natural gas (98% methane) supplied from wells in the southeastern USA and was selected on the basis of high purity (food grade) and a $\delta^{13}\text{C}$ value of $-52 \pm 1\text{‰}$. The $\delta^{13}\text{C}$ content of the CO₂ under which plant growth occurred was measured during the experiment. The pure CO₂ from the supply tank used to generate the CO₂ enrichment was sampled after each delivery of new CO₂ gas. The averaged $\delta^{13}\text{C}$ content over the two years was $-52.4 \pm 0.5\text{‰}$. The air in the chambers were also measured and resulted in an average $\delta^{13}\text{C}$ content of $-8.5 \pm 0.9\text{‰}$ for the ambient chambers (ambient air only) and $-33.0 \pm 1.4\text{‰}$ for the CO₂ enriched chambers (mixture of pure CO₂ and air).

The $\delta^{13}\text{C}$ of plant tissue depends on the $\delta^{13}\text{CO}_2$ content of the air in which plants are growing and the photosynthetic pathway of the plants (C3 or C4). The C3 photosynthesis pathway discriminates against $^{13}\text{CO}_2$ resulting in lower $\delta^{13}\text{C}$ values compared to plants with the C4 photosynthesis pathway. The $\delta^{13}\text{CO}_2$ of air for the elevated CO₂ treatment ($\delta^{13}\text{C} = -33.0\text{‰}$) was lower compared to the ambient CO₂ treatment ($\delta^{13}\text{C} = -8.5\text{‰}$). As a result, four distinct levels of root $\delta^{13}\text{C}$ were measured in our study (Table 1). Within these four groups, the root $\delta^{13}\text{C}$ content was consistent.

The original soil organic matter C content was very low with the C concentration ranging from 0.24 to 0.26%, and the $\delta^{13}\text{C}$ content of the initial soil ranged from -22.4 to -24.1‰ (Table 2). Most of the initial soil C was in the MinC associated fraction, with an average MinC

Table 2 Initial percentage C and $\delta^{13}\text{C}$ of soil organic matter carbon (SOMC), mineral associated organic matter carbon (MinC), and charcoal†.

Depth (cm)	SOMC			MinC			Charcoal		
	Percent C %	$\delta^{13}\text{C}$ ‰	Total C Mg ha ⁻¹	Percent C %	$\delta^{13}\text{C}$ ‰	Total C Mg ha ⁻¹	Percent C %	$\delta^{13}\text{C}$ ‰	Total C Mg ha ⁻¹
0–5	0.26 (0.01)	–22.4 (0.55)	190 (6)	0.22 (0.003)	–23.8 (0.06)	159 (2)	0.04 (0.01)	–25.4 (0.35)	30 (5)
5–10	0.25 (0.01)	–22.6 (0.68)	185 (3)	0.21 (0.003)	–23.8 (0.06)	161 (4)	0.03 (0.003)	–25.5 (0.36)	24 (2)
10–15	0.25 (0.02)	–22.9 (0.62)	195 (10)	0.21 (0.004)	–23.8 (0.06)	166 (3)	0.04 (0.01)	–25.5 (0.20)	29 (7)
15–30	0.24 (0.02)	–24.1 (0.18)	519 (33)	0.22 (0.01)	–23.8 (0.05)	470 (10)	0.02 (0.02)	–25.0 (0.60)	49 (32)

†Values represent means of three replicates. Standard deviation given in parentheses.

concentration ranging from 0.21 to 0.22% compared to a concentration of 0.02–0.04% for charcoal. The initial $\delta^{13}\text{C}$ for the soil indicates that this soil developed in an ecosystem dominated by C_3 species (Boutton 1991b). The $\delta^{13}\text{C}$ determination of the C fractions resulted in distinct $\delta^{13}\text{C}$ levels for MinC and charcoal that were very consistent with depth, with an average $\delta^{13}\text{C}$ of –23.8‰ for MinC and –25.5‰ for charcoal (Table 2). Differences in $\delta^{13}\text{C}$ levels between the MinC and charcoal fractions compared to the SOMC $\delta^{13}\text{C}$ levels indicates that not all of the C present in the POMC fraction was accounted for in the $\delta^{13}\text{C}$ analysis of the charcoal.

The $\delta^{13}\text{C}$ content of root tissue was sufficiently different from the initial soil SOMC and MinC $\delta^{13}\text{C}$ content to allow tracking of new C originating from the crops into the soil. Isotopic mass balance methods (Balesdent *et al.* 1988; Leavitt *et al.* 1994) and the following equation were utilized:

$$\delta^{13}\text{C}_{\text{soil}} = f_{\text{input}}(\delta^{13}\text{C}_{\text{input}}) + f_{\text{soil original}}(\delta^{13}\text{C}_{\text{soil original}}),$$

where $\delta^{13}\text{C}_{\text{soil}}$ is the $\delta^{13}\text{C}$ content of the soil C samples, $\delta^{13}\text{C}_{\text{input}}$ is the $\delta^{13}\text{C}$ content of new plant biomass input, $\delta^{13}\text{C}_{\text{soil original}}$ is the original $\delta^{13}\text{C}$ content of soil C measured initially, f_{input} is the fraction of soil C originating from the new crop production, and $f_{\text{soil original}}$ is the fraction of soil C originally in the soil before initiation of the study. The $\delta^{13}\text{C}$ content of root material from each plot was used for $\delta^{13}\text{C}_{\text{input}}$ in 1992, and the average $\delta^{13}\text{C}$ content of roots from 1992 and 1993 from each plot was used for $\delta^{13}\text{C}_{\text{input}}$ in 1993.

Calculations of new carbon are not presented on the POMC fraction because of the potential complications involved with charcoal and missed C in the POMC fraction. Soil C contents were summed over all soil depths to calculate the total C content to 30 cm for the SOMC and the MinC soil carbon fraction. The new soil content to a depth of 30 cm was calculated by multiplying the fraction of new C in SOMC and MinC fraction by its C content at each depth and summing across soil depths.

Statistical analysis of data was performed using the

GLM procedure and means were separated using contrast statements and least significant difference (LSD) at an *a priori* 0.10 probability level (SAS Institute 1985). The experimental design was a split-plot with three replications. The term '*trend*' is used to designate appreciable, but nonsignificant, treatment effects with probability of greater *F*-values between 0.10 and 0.25 probability levels. The term '*new carbon*' is used to designate that portion of carbon that was isotopically determined to be from carbon originating from the plant inputs following the initiation of the study. No significant difference was observed between the open plot (no chamber) treatments and the ambient CO_2 treatments for the measurements discussed in this manuscript therefore no discussion of the open plot treatments was included.

Results

Elevated atmospheric CO_2 effect on plants

For both plant species, significant differences were observed in the mass of plant residue returned to the plots (root biomass and above-ground biomass minus seed) at harvest as a function of atmospheric CO_2 treatment during both years of the study (Table 3). Total C returned to the soil in residue significantly increased with elevated CO_2 in both years (Table 3). Similar to total biomass input, in 1992 grain sorghum residue C was significantly higher than soybeans residue. In 1993, unlike the biomass inputs, no significant difference was observed between the two species, although a trend ($P = 0.18$) was observed for soybean to have higher C compared to grain sorghum. In 1993, the total input of residue C was greatly increased compared to 1992 in both grain sorghum and soybean. In addition, the impact of elevated CO_2 compared to ambient CO_2 was greatly increased in 1993 in contrast with 1992.

Differences were also observed in the C:N ratio of plant residues returned to soil after harvest (Table 3). Total N returned to the plots in residue was much greater in soybean compared to grain sorghum in both years,

Table 3 Total dry weight, total C content, and C:N ratio of crop residue (root biomass and above-ground biomass – seed) grown under ambient or elevated (twice ambient) atmospheric CO₂ concentrations returned to soil after harvest in 1992 and 1993†.

Crop	Total Dry Weight (Mg ha ⁻¹)			Total C (g m ⁻²)			C:N		
	Ambient	Elevated	Mean	Ambient	Elevated	Mean	Ambient	Elevated	Mean
1992									
Grain Sorghum	4.7	6.5	5.6 a	206	293	250 a	116.3	122.7	119.5 a
Soybean	4.3	6.2	5.2 b	194	280	240 b	21.7	26.8	24.2 b
Mean	4.5 a	6.3 b		200 a	287 b		69.0 a	74.8 a	
1993									
Grain Sorghum	7.4	10.3	8.9 a	311	449	380 a	111.4	136.2	123.8 a
Soybean	8.1	12.3	10.2 b	358	532	445 a	18.9	21.1	20.0 b
Mean	7.8 a	11.3 b		335 a	490 b		65.1 a	78.6 b	

†Values represent means of three replicates. Values within a row or within a column followed by the same letter do not differ significantly (0.10 level).

Table 4 The effect of plant species and atmospheric CO₂ content on soil organic matter carbon δ¹³C content in 1992 and 1993 (‰)†.

Depth (cm)	Grain Sorghum		Soybean	
	Ambient	Elevated	Ambient	Elevated
1992				
0–5	–22.4	–23.8	–22.9	–24.2
5–10	–22.7	–23.3	–22.8	–23.8
10–15	–23.2	–24.7	–23.3	–23.7
15–30	–24.1	–24.1	–24.1	–24.1
1993				
0–5	–22.9	–24.8	–25.0	–27.0
5–10	–22.1	–24.6	–24.2	–25.4
10–15	–23.9	–24.8	–27.7	–25.7
15–30	–24.0	–24.6	–24.5	–25.0

†Values represent means of three replicates.

presumably because soybean is a legume with symbiotic N₂-fixation capabilities involving *Rhizobium japonicum*. This resulted in a significant difference for the C:N ratio between crop species for both years, with grain sorghum having a higher C:N ratio than soybean (Table 3). In 1993, the C:N ratio was also affected by the CO₂ treatment, with the ambient CO₂ having a lower C:N ratio than the elevated CO₂ treatment (Table 3). A similar trend ($P = 0.12$) was observed in 1992 for the C:N ratio to be higher for the elevated CO₂ treatment.

Soil carbon

The δ¹³C content of soil samples indicated that new inputs of C into the soil system shifted the δ¹³C content of both the SOMC and the MinC (Tables 4 and 5). Changes in the δ¹³C from the initial levels were observed for both crop species and for CO₂ treatments at all four soil depths in 1992 for both SOMC (except 15–30 cm) and MinC (Tables 4 and 5). Likewise, δ¹³C content of SOMC and

Table 5 The effect of plant species and atmospheric CO₂ content on soil mineral associated organic matter carbon δ¹³C content in 1992 and 1993 (‰)†.

Depth (cm)	Grain Sorghum		Soybean	
	Ambient	Elevated	Ambient	Elevated
1992				
0–5	–23.5	–24.3	–24.1	–24.8
5–10	–23.7	–24.0	–23.9	–24.3
10–15	–23.7	–23.9	–23.8	–24.0
15–30	–23.6	–23.9	–24.0	–23.9
1993				
0–5	–23.1	–25.1	–24.6	–27.4
5–10	–23.4	–24.6	–24.2	–25.2
10–15	–23.6	–24.4	–24.1	–24.9
15–30	–23.7	–24.2	–23.9	–24.4

†Values represent means of three replicates.

MinC was changed in 1993 in respect to both initial δ¹³C content and the δ¹³C content measured in 1992 at all four soil depths (Tables 4 and 5).

In 1993, SOMC content in all treatments was increased compared to 1992 and initial SOMC content (Tables 2 and 6). Soils samples were taken at harvest in 1992, and therefore, because crop residues had not been returned, above-ground biomass would have little impact in this year. Soil sampling in 1993 would include the impact of above-ground biomass from the 1992 growing season and the root biomass from both years crop production. However, no significant treatment effects were observed in SOMC in either year.

In 1992, no significant treatment effects were observed in the total C content of the MinC fraction (Table 7), but in 1993, the total C content of MinC fraction was generally increased compared to 1992 levels and significant treatment effects were observed (Table 7). In this year, the MinC content was significantly increased in grain

Table 6 The effect of plant species and atmospheric CO₂ content on total soil organic carbon content and soil new carbon content (g m⁻², isotopically determined)†.

Crop	Total			New		
	Ambient	Elevated	Mean	Ambient	Elevated	Mean
1992						
Grain Sorghum	1053	1004	1034 a	10.7	87.5	46.3 a
Soybean	1015	1077	1048 a	54.7	34.7	52.5 a
Mean	1034 a	1041 a		32.7 a	61.1 a	
Contrast						
A vs. E sorghum		NS			NS	
A vs. E soybean		NS			0.05	
1993						
Grain Sorghum	1224	1193	1208 a	28.8	161.9	67.9 a
Soybean	1172	1204	1208 a	291.4	120.0	221.8 b
Mean	1198 a	1198 a		160.1 a	140.9 b	
Contrast						
A vs. E sorghum		NS			0.001	
A vs. E soybean		NS			0.003	

†Values represent means of three replicates. Values within a row or within a column followed by the same letter do not differ significantly (0.10 level).

Table 7 The effect of plant species and atmospheric CO₂ content on total soil mineral associated organic matter carbon content and soil new soil mineral associated organic matter carbon content (g m⁻², isotopically determined)†.

Crop	Total			New		
	Ambient	Elevated	Mean	Ambient	Elevated	Mean
1992						
Grain Sorghum	909	922	917 a	14.7	22.0	16.6 a
Soybean	875	906	898 a	29.2	13.0	24.0 a
Mean	892 a	914 a		22.0 a	17.5 a	
Contrast						
A vs. E sorghum		NS			0.25	
A vs. E soybean		NS			NS	
1993						
Grain Sorghum	935	962	950 a	26.3	65.8	43.1 a
Soybean	873	948	922 b	70.7	58.8	70.7 a
Mean	904 a	955 b		48.5 a	62.3 a	
Contrast						
A vs. E sorghum		0.03			NS	
A vs. E soybean		NS			0.07	

†Values represent means of three replicates. Values within a row or within a column followed by the same letter do not differ significantly (0.10 level).

sorghum compared to soybean and by elevated CO₂ compared to ambient CO₂, with the difference due to a significant increase in elevated CO₂ in grain sorghum (contrast_{A vs. E sorghum} = 0.03).

The new C content in the SOMC and MinC pools calculated from shifts in $\delta^{13}\text{C}$ are shown in Tables 6 and 7, with new C found in both the SOMC and the MinC in both years and with more new C observed in 1993 compared to 1992. The amount of new C found in SOMC and MinC depended on both crop species and the CO₂ environment in which plants were growing. In 1992,

while no significant difference was observed for new C in SOMC for grain sorghum, new C in SOMC was significantly lower in soybeans under elevated CO₂ compared to ambient CO₂ (Table 6). While not significant, means for new C in MinC were similar to differences observed with new C in SOMC (Table 7).

In 1993, more new C was observed in SOMC with grain sorghum in the elevated CO₂ treatment compared to ambient CO₂ (Table 6). This is consistent with increased C inputs from crop residue (Table 3). With soybean, more new C in SOMC was found with the ambient CO₂

treatment compared to elevated CO₂ (Table 6). Similarly, in 1993 the amount of new C in the MinC fraction was significantly lower in elevated CO₂ compared to ambient CO₂ with soybeans (Table 7). This was not consistent with the levels of residue C input into the soil from soybeans (Table 3).

This pattern did not agree with the observed C levels of total SOMC and MinC in the soil, which indicated no significant difference between crops and CO₂ treatments. In fact, treatment means indicated increased storage of MinC with elevated CO₂ with soybean (especially in 1993), which is consistent with the levels of C input from residue measured in this study.

Discussion

Several contradictory hypotheses addressing the potential for C storage in terrestrial ecosystems have been forwarded (Goudriaan & de Ruiter 1983; Lamborg *et al.* 1983; Bazzaz 1990; Lekkerkerk *et al.* 1990). To date, none of these hypotheses has been proven, and the $\delta^{13}\text{C}$ data from this study indicate that some of the different mechanisms described in these hypothesis, while seemingly contradictory, may actually occur concurrently depending on the plant species involved.

Bazzaz (1990) has argued that decomposition rates in soil with elevated CO₂ may be slower due to the increased C:N ratio of plant residue grown under elevated CO₂ conditions, while Lamborg *et al.* (1983) have argued that increased soil microbial activity due to greater biomass C inputs under elevated CO₂ could lead to increased soil organic matter decomposition ('the priming effect') and therefore atmospheric CO₂ enrichment would not result in accumulation of soil organic C. There was evidence of a priming effect in SOMC measured with soybean. While the soybeans with elevated CO₂ had higher biomass inputs, more new C was observed with ambient CO₂ compared to elevated CO₂, but no change in SOMC after 2 years (Table 6). However, there was no evidence of a priming effect with grain sorghum, with more new carbon with elevated CO₂ for both SOMC and MinC and more total MinC in 1993 (Tables 6 and 7). These results are consistent with results reported by Prior *et al.* (1997a) for short-term CO₂ efflux from this study, which indicated that elevated CO₂ increased CO₂ efflux under soybeans but not with grain sorghum.

With grain sorghum in 1993, MinC and the calculated new C in MinC indicated increased soil C storage. Since most of the soil C was found in this C pool, this would indicate that increased soil storage of C may occur over the long-term. This agrees with the hypothesis that increased biomass inputs into soil (due to increased plant growth and higher C:N ratio with elevated CO₂, Table 3) will increase soil C storage (Bazzaz 1990).

With soybean, elevated CO₂ had no significant effect on soil C (although means were higher), and the calculated new C in elevated CO₂ was decreased compared to ambient CO₂ in both SOMC and MinC. This is consistent with the hypotheses of Goudriaan & de Ruiter (1983) which proposed that increased soluble, easily decomposed C inputs as a consequence of higher atmospheric CO₂ could accentuate the substrate preferences of soil microbes and a preference for easily decomposable substrates would retard the decomposition of plant debris and native soil organic matter. This hypothesis is also consistent with data reported by Lekkerkerk *et al.* (1990) using ^{14}C techniques, who found that the input of easily decomposable root-derived material in the soil of wheat plants was increased but, due to microbial preference for these materials, turnover of more resistant soil organic matter was reduced under elevated CO₂. With soybean, there was evidence of increased residue decomposition under elevated CO₂ conditions, with a reduction in the accumulation of new C. However, this increase in new C decomposition with elevated CO₂ was apparently accompanied by reduction in original SOMC decomposition (Tables 6 and 7).

A partial explanation of the reduction in the calculated new C in soybeans may be from shifts of $\delta^{13}\text{C}$ content due to discrimination during decomposition process. Boutton (1991b) reported that the $\delta^{13}\text{C}$ content of lignin is often 2–6‰ depleted compared to whole plant tissue $\delta^{13}\text{C}$. Since lignin is resistant to decomposition, SOMC $\delta^{13}\text{C}$ content could be depleted due to the decomposition process, especially in this low SOMC soil. This effect could also be exaggerated with the ambient soybean treatment since the difference in the initial soil $\delta^{13}\text{C}$ content and $\delta^{13}\text{C}$ content of root tissue is closer compared to $\delta^{13}\text{C}$ of roots in the other treatments.

The difference between new C storage compared to total C storage clearly indicates that shifts have occurred in the decomposition of SOMC due both to crop species and the CO₂ environment in which they were grown. It also indicates that shifts in the decomposition of original SOMC occurred with the change in crop species and atmospheric CO₂ level.

This difference in plant species for plant decomposition mechanisms could likely be driven by N availability. Green *et al.* (1995) reported that additions of NO₃⁻ following corn production promoted corn residue decomposition but suppressed C mineralization from SOMC. Changes in the mechanism of soil C storage in agroecosystems due to species effects have been noted by Prior *et al.* (1997b). They reported that soil under free-air CO₂ enrichment which had three years of cotton production (Wood *et al.* 1994) and two years of wheat production (Prior *et al.* 1997b) showed higher levels of organic C, but that the decomposition processes may

have been different between the two plant species. Our data suggest that the potential for a priming effect could be species dependent; differences are likely due to changes in the C:N ratio and other quality factors of plant residue and the cycling of N in soil. These speculations agree with findings of Torbert *et al.* (1995) for the decomposition of cotton residue produced under elevated CO₂ conditions. Differences in decomposition between cotton plant parts were due to changes in C:N ratio, but not due to changes in C:N ratio derived from the elevated CO₂ effect. In their study, the rate of plant decomposition was driven by the intrinsic ability of the soil to support microbial activity. The change in decomposition of the plant material may have also been affected by changes in other plant nutrients in the soil and to changes in soil moisture status due to the treatments.

Our data suggest that elevated CO₂ may result in increased storage of C in soil over long-term, but that the mechanisms for storage may be different for different plant species. With grain sorghum, the high C:N ratio led to slow microbial decomposition resulting in increased new soil C, but storage of C from elevated CO₂ was increased only in the MinC fraction. With soybeans, the low C:N ratio promoted microbial decomposition of new biomass inputs, however, the increased decomposition of new C inputs in elevated CO₂ apparently reduced the decomposition of old C, resulting in a trend for increased soil storage of C.

Acknowledgements

The authors are indebted to Barry G. Dorman, Auburn University, and Larry Giles, Duke University. Support from Global Change Research, Environmental Sciences Division, U.S. Department of Energy (DE-AI05-95ER62088) and the Alabama Agricultural Experiment Station, Auburn University (3-955028) gratefully acknowledged.

References

- Ajtay GL, Ketner P, Duvigneaud P (1979) Terrestrial primary production and phytomass. In: *The Global Carbon Cycle* (eds Bolin B, Degens ET, Kempe S, Ketner P), pp. 129–181. John Wiley, New York.
- Amthor JS (1991) Respiration in a future, higher-CO₂ world. *Plant, Cell and Environment*, **14**, 13–20.
- Amthor JS, Mitchell RJ, Runion GB, Rogers HH, Prior SA, Wood CW (1994) Energy content and construction costs of plants grown in elevated CO₂. *New Phytologist*, **128**, 443–450.
- Balesdent J, Wagner GH, Mariotti A (1988) Soil organic matter turnover in long-term field experiments as revealed by carbon-13 natural abundance. *Soil Science Society of America Journal*, **52**, 118–124.
- Bazzaz FA (1990) The response of natural ecosystems to the rising global CO₂ levels. *Annual Review of Ecology and Systematics*, **21**, 167–196.
- Bohm W (1979) *Methods for Studying Root Systems*. Springer-Verlag, New York.
- Boutton TW (1991a) Stable carbon isotope ratios of natural materials: I. sample preparation and mass spectrometric analysis. In: *Carbon Isotope Techniques* (eds Coleman DC, Fry B), Academic Press, San Diego, CA, 274pp.
- Boutton TW (1991b) Stable carbon isotope ratios of natural materials: II. Atmospheric, terrestrial, marine, and freshwater environments. In: *Carbon Isotope Techniques* (eds Coleman DC, Fry B), Academic Press, San Diego, CA, 274pp.
- Cambardella CA, Elliott ET (1992) Particulate soil organic-matter changes across a grassland cultivation sequence. *Soil Science Society of America Journal*, **56**, 777–783.
- Fisher MJ, Rao RM, Ayarza MA, Lascano CE, Sanz JI, Thomas RJ, Vera RR (1994) Carbon storage by introduced deep-rooted grasses in the South American savannas. *Nature*, **371**, 236–238.
- Goudriaan J, de Ruiter HE (1983) Plant growth in response to CO₂ enrichment at two levels of nitrogen and phosphorus supply. 1. Dry matter, leaf area, and development. *Netherlands Journal of Agricultural Science*, **31**, 157–169.
- Goudriaan J, Zadoks JC (1995) Global climate change: Modelling the potential responses of agro-ecosystems with special reference to crop protection. *Environmental Pollution*, **87**, 215–224.
- Green CJ, Blackmer AM, Horton R (1995) Nitrogen effects on conservation of carbon during corn residue decomposition in soil. *Soil Science Society of America Journal*, **59**, 453–459.
- Jackson RBIV (1992) On estimating agriculture's net contribution to atmospheric carbon. *Water, Air and Soil Pollution*, **64**, 121–137.
- Keeling CD, Bacastow RB, Carter AF, *et al.* (1989) A three dimensional model of atmospheric CO₂ transport based on observed winds: observational data and preliminary analysis. In: *Aspects of Climate Variability in the Pacific and the Western Americas* (ed. Peterson DH). *Geophysical Monograph*, **55**, 165–235.
- Kern JS, Johnson MG (1993) Conservation tillage impacts on national soil and atmospheric carbon levels. *Soil Science Society of America Journal*, **57**, 200–210.
- Lamborg MR, Hardy WF, Paul EA (1983) Microbial effects. In: *CO₂ and Plants: the Response of Plants to Rising Levels of Atmospheric CO₂* (ed. Lemon ER), pp. 131–176. American Association of Advanced Science Selected Symposium, Washington, DC.
- Leavitt SW, Paul EA, Kimball BA, *et al.* (1994) Carbon isotope dynamics of free-air CO₂-enriched cotton and soils. *Agriculture, Forestry and Meteorology*, **70**, 87–101.
- van de Lekkerkerk LJA Geijn SC, Van Veen JA (1990) Effects of elevated atmospheric CO₂ levels on the carbon economy of a soil planted with wheat. In: *Soils and the Greenhouse Effect* (ed. Bouwman AF), pp. 423–429. John Wiley, New York.
- Liljeroth E, Kuikman P, Van Veen JA (1994) Carbon translocation to the rhizosphere of maize and wheat and influence on turnover of the native soil organic matter at different soil N levels. *Plant Soil*, **161**, 233–240.

- Parton WJ, Schimel DS, Cole CV, Ojima DS (1986) Analysis of factors controlling soil organic matter levels in Great Plains grasslands. *Soil Science Society of America Journal*, **51**, 1173–1179.
- Post WM, Emanuel WR, King AW (1992) Soil organic matter dynamics and the global carbon cycle. In: *World Inventory of Soil Emission Potentials* (eds Batjes NH, Bridges EM), International Soil Reference Information Center, Wageningen, The Netherlands.
- Post WM, Peng TH, Emanuel WR, King AW, Dale VH, DeAngelis DL (1990) The global carbon cycle. *American Scientist*, **78**, 310–326.
- Prior SA, Rogers HH, Runion GB, *et al.* (1995) Free-air CO₂ enrichment of cotton: Root morphological characteristics. *Journal of Environmental Quality*, **24**, 678–683.
- Prior SA, Rogers HH, Runion GB, Torbert HA, Reicosky DC (1997a) CO₂ enriched agro-ecosystems: influence of tillage on short-term soil CO₂ efflux. *Journal of Environmental Quality*, **26**, 244–252.
- Prior SA, Torbert HA, Runion GB, *et al.* (1997b) Free-air CO₂ enrichment of wheat: Soil carbon and nitrogen dynamics. *Journal of Environmental Quality*, **26**, 1161–1166.
- Reeves DW, Rogers HH, Prior SA, Wood CW, Runion GB (1994) Elevated atmospheric carbon dioxide effects on sorghum and soybean nutrient status. *Journal of Plant Nutrition*, **17**, 1939–1954.
- Rogers HH, Heck WW, Heagle AS (1983) A field technique for the study of plant responses to elevated carbon dioxide concentrations. *Air Pollution Control Association Journal*, **33**, 42–44.
- Rogers HH, Dahlman RC (1991) Crop responses to CO₂ enrichment. *Vegetatio*, **104/105**, 117–131.
- Rogers HH, Runion GB, Krupa SV (1994) Plant responses to atmospheric CO₂ enrichment with emphasis on roots and the rhizosphere. *Environmental Pollution*, **83**, 155–189.
- SAS Institute Inc. (1985) *Sas User's Guide: Statistics*. Statistical Analysis System (SAS) Institute, Inc., Cary, NC, 956pp.
- Schimel D, Enting IG, Heimann M, Wigley TML, Raynaud D, Alves D, Siegenthaler U (1995) CO₂ and the carbon cycle. In: *Climate Change 1994* (eds Houghton JT, Meira Filho LG, Bruce J *et al.*), pp. 35–71. Cambridge University Press, Cambridge.
- Schlesinger WH (1984) Soil organic matter: A source of atmospheric CO₂. In: *The Role of Terrestrial Vegetation in the Global Carbon Cycle* (ed. Woodwell GM), pp. 111–127. John Wiley, New York.
- Schlesinger WH (1986) Changes in soil carbon storage and associated properties with disturbance and recovery. In: *The Changing Carbon Cycle: A Global Analysis* (eds Trabalka JR, Reichle DE), pp. 194–220. Springer-Verlag, New York.
- Schlesinger WH (1990) Evidence from chronosequence studies for a low carbon-storage potential of soils. *Nature*, **348**, 232–234.
- Tans PP, Fung IY, Takahashi T (1990) Observational constraints on the global atmospheric CO₂ budget. *Science*, **247**, 1431–1438.
- Thomas JF, Harvey CN (1983) Leaf anatomy of four species grown under continuous CO₂ enrichment. *Botanical Gazette*, **144**, 303–309.
- Torbert HA, Prior SA, Rogers HH (1995) Elevated atmospheric CO₂ effects on cotton plant residue decomposition. *Soil Science Society of America Journal*, **59**, 1321–1328.
- Wallace A, Wallace GA, Cha JW (1990) Soil organic matter and the global carbon cycle. *Journal of Plant Nutrition*, **13**, 459–466.
- Wilson AT (1978) Pioneer agriculture explosion and CO₂ levels in the atmosphere. *Nature*, **273**, 40–41.
- Wood CW, Torbert HA, Rogers HH, Runion GB, Prior SA (1994) Free-air CO₂ enrichment effects on soil carbon and nitrogen. *Agriculture, Forestry and Meteorology*, **70**, 103–116.